

CLAIMS

1. A method for determining susceptibility of a thymus in a patient to activation through disruption of sex steroid signaling to the thymus.

2. The method of claim 1 wherein the method of disrupting the sex steroid signaling to the thymus is through surgical castration to remove the patient's gonads.

3. The method of claim 1 wherein the method of disrupting the sex steroid signaling to the thymus is through administration of one or more pharmaceuticals.

4. The method of claim 3 wherein the pharmaceuticals are selected from the group consisting of LHRH analogs, anti-LHRH receptor antibodies, anti-LHRH vaccines and combinations thereof.

5. The method of claim 4 wherein the LHRH analogs are selected from LHRH agonists and LHRH antagonists.

6. The method of claim 5 wherein the LHRH analogs are selected from the group consisting of Eulexin, Goserelin, Leuprolide, Dioxalan derivatives, Triptorelin, Meterelin, Buserelin, Histrelin, Nafarelin, Lutrelin, Leuprorelin, Deslorelin, Abarelix, Cetrorelix, Zoladex and Leupron.

7. The method of claim 3 wherein the patient's thymus has been at least in part deactivated.

8. The method of claim 3 wherein the patient is post-pubertal.

9. The method of claim 5 wherein the LHRH antagonists are quick-acting LHRH antagonists.

10. The method of claim 9 wherein the LHRH antagonists are selected from the group consisting of Abarelix and Cetrorelix.

11. The method of claim 4 wherein reduction is induced by administration of one or more LHRH agonists and one or more LHRH antagonists.

12. The method of claim 1 comprising the step of monitoring the concentration of one or more thymopoietic cytokines in the patient's blood and/or plasma.

13. The method of claim 12 wherein the cytokine is Interleukin-7.

14. The method of claim 12 comprising the steps of

- 5 a. obtaining a blood sample from a patient prior to the disruption;
- b. obtaining at least one blood sample from a patient subsequent to the disruption;
- c. measuring the amount of thymopoietic cytokine(s) present in each sample; and
- 10 d. comparing the amounts of thymopoietic cytokine(s) in the samples to each other,

such that an early increase in thymopoietic cytokine(s) in the patient after disruption indicates activation of the patient's thymus.

15 15. The method of claim 14 wherein the increase occurs within about one week of disruption.

16. The method of claim 14 wherein the increase occurs within about 4 to 5 days of disruption.

17. The method of claim 14 wherein the increase occurs within about 2 to 3 days of disruption.

20 18. The method of claim 14 wherein the increase occurs within about 24 hours of disruption.

19. The method of claim 1 comprising the step of monitoring the concentration of one or more thymopoietic hormones in the patient's blood and/or plasma.

20. The method of claim 19 wherein the hormone is selected from the group consisting of thymosin, thymulin and FTS.

21. The method of claim 19 comprising the steps of

- a. obtaining a blood sample from a patient prior to the disruption;
- b. obtaining at least one blood sample from a patient subsequent to the disruption;
- c. measuring the amount of thymopoietic hormone(s) present in each sample; and
- d. comparing the amounts of thymopoietic hormone(s) in the samples to each other,

such that an increase in thymopoietic hormone(s) in the patient after disruption indicates activation of the patient's thymus.

22. The method of claim 21 wherein the increase occurs within about one week of disruption.

23. The method of claim 21 wherein the increase occurs within about 4 to 5 days of disruption.

24. The method of claim 21 wherein the increase occurs within about 2 to 3 days of disruption.

25. The method of claim 21 wherein the increase occurs within about 24 hours of disruption.

26. A method of identifying thymic factors comprising the steps of

- a) obtaining a blood sample from a patient;
- b) disrupting sex steroid mediated signaling to the patient's thymus;

- c) obtaining at least one blood sample from the patient after disruption;
- d) performing protein analysis on each sample;
- e) identifying new proteins that are found in the samples taken after disruption and in lesser concentrations or not at all in samples taken before disruption.

5 27. The method of claim 26 wherein the blood samples are treated to separate out the plasma and the analyses are performed on the plasma samples.

 28. The method of claim 27 wherein the plasma samples are subjected to two dimensional gel electrophoresis.

10 29. The method of claim 1 comprising the step of monitoring the production of new T cells in the patient's blood.

 30. The method of claim 29 wherein the production of new T cells is monitored by detecting the presence in these cells of TRECs.

 31. The method of claim 30 comprising the steps of

- a) sampling the patient's blood before and after inhibition;
- 15 b) sorting the cells in samples to obtain an enhanced population of T cells;
- c) isolating the DNA of the cells in the samples; and
- d) performing PCR on the isolated DNA using primers specific for TRECs.

 32. The method of claim 31 wherein the PCR primers are selected from the group consisting of DNA SEQ ID NO:1, DNA SEQ ID NO:2, DNA SEQ ID NO:3 and DNA SEQ ID
20 NO:4.

 33. The method of claim 31 wherein an increase in TRECs after inhibition indicates thymic activation.

34. The method of claim 33 wherein the increase occurs within about one week of disruption.

35. The method of claim 33 wherein the increase occurs within about 4 to 5 days of disruption.

5 36. The method of claim 33 wherein the increase occurs within about 2 to 3 days of disruption.

37. The method of claim 33 wherein the increase occurs within about 24 hours of disruption.

10 38. A method for determining the susceptibility of an at least partially atrophied thymus to reactivation in a patient, comprising:

monitoring the level in the patient's blood or serum of one or more markers associated with activation of the thymus,

disrupting sex steroid-mediated signaling to the thymus of the patient,

monitoring the level in the patient's blood or serum of the one or more markers, and

15 comparing the level of the one or more markers before and after disruption of sex steroid-mediated signaling,

wherein an early increase in the level of any one of the markers following disruption of sex steroid-mediated signaling indicates susceptibility of the patient's thymus to reactivation.

20 39. The method of claim 38, wherein the patient has a disease that at least in part atrophied the thymus of the patient.

40. The method of claim 38, wherein the patient has had a treatment of a disease, wherein the treatment at least in part atrophied the thymus of the patient.

41. The method of claim 40, wherein the treatment is immunosuppression, chemotherapy, or radiation treatment.

42. The method of claim 38, wherein the patient is post-pubertal.

43. The method of claim 38, wherein the sex steroid-mediated signaling to the thymus is disrupted by surgical castration.

44. The method of claim 38, wherein the sex steroid-mediated signaling to the thymus
5 is disrupted by chemical castration.

45. The method of claim 38, wherein the sex steroid-mediated signaling to the thymus is disrupted by administration of one or more pharmaceuticals.

46. The method of claim 45, wherein the one or more pharmaceuticals is selected from the group consisting of LHRH agonists, LHRH antagonists, anti-LHRH vaccines, anti-
10 androgens, anti-estrogens, SERMs, SARMs, SPRMs, ERDs, aromatase inhibitors, anti-progestogens, and combinations thereof.

47. The method of claim 46, wherein the LHRH agonists are selected from the group selected from the group consisting of Eulexin, Goserelin, Leuprolide, Dioxalan derivatives, Triptorelin, Meterelin, Buserelin, Histrelin, Nafarelin, Lutrelin, Leuprorelin, Deslorelin,
15 Cystorelin, Decapeptyl, Gonadorelin, and combinations thereof.

48. The method of claim 46, wherein the LHRH antagonists are selected from the group consisting of Abarelix, Cetrorelix, and combinations thereof.

49. The method of claim 46, wherein the one or more pharmaceuticals are a combination of LHRH agonists and LHRH antagonists.

20 50. The method of claim 38, wherein the early increase occurs within four weeks following disruption of sex steroid-mediated signaling.

51. The method of claim 38, wherein the early increase occurs within two weeks following disruption of sex steroid-mediated signaling.

25 52. The method of claim 38, wherein the early increase occurs within one week following disruption of sex steroid-mediated signaling.

53. The method of claim 38, wherein the early increase occurs within about 4 to 5 days following disruption of sex steroid-mediated signaling.

54. The method of claim 38, wherein the early increase occurs within about 2 to 3 days following disruption of sex steroid-mediated signaling.

5 55. The method of claim 38, wherein the early increase occurs within about 24 hours following disruption of sex steroid-mediated signaling.

56. The method of claim 38, wherein the marker is a thymopoietic hormone or thymopoietic cytokine.

10 57. The method of claim 56, wherein the marker is IL-7, Factor Thymique Serique (FTS) or thymulin.

58. The method of claim 38, wherein the marker is a thymosin.

59. The method of claim 58, wherein the thymosin is thymosin-alpha 1 or thymosin-beta 4.

60. The method of claim 38, wherein the marker is thymopoietin.

15 61. A method for determining the susceptibility of an at least partially atrophied thymus to reactivation in a patient, comprising:

monitoring the *in vitro* responsiveness of T cells in the patient's blood or serum to anti-CD3 cross-linking, wherein responsiveness is determined by monitoring proliferation of the T cells,

20 disrupting sex steroid-mediated signaling to the thymus of the patient,

monitoring the *in vitro* responsiveness of the T cells in the patient's blood or serum to anti-CD3 cross-linking, wherein responsiveness is determined by monitoring proliferation of the T cells, and

comparing the *in vitro* responsiveness of the T cells in the patient's blood or serum before and after disruption of sex steroid-mediated signaling to the thymus of the patient,

wherein an early increase in the *in vitro* responsiveness of the T cells to anti-CD3 cross-linking following disruption of sex steroid-mediated signaling indicates susceptibility of the patient's thymus to reactivation.

62. A method for determining the susceptibility of an at least partially atrophied thymus to reactivation in a patient, comprising:

monitoring the level of newly produced T cells in the patient's blood or serum,

disrupting sex steroid-mediated signaling to the thymus of the patient,

10 monitoring the level of newly produced T cells in the patient's blood or serum, and

comparing the level of the newly produced T cells in the patient's blood or serum before and after disruption of sex steroid-mediated signaling,

wherein an early increase in the level of the newly produced T cells following disruption of sex steroid-mediated signaling indicates susceptibility of the patient's thymus to reactivation.

15 63. The method of claim 62, wherein the monitoring of the level of newly produced T cells is accomplished by monitoring Ki67, CD62L, CD45RA, or combinations thereof.

64. The method of claim 63, wherein the monitoring of the level of newly produced T cells is accomplished by monitoring T Cell Receptor Excision Circles (TRECs).

20 65. A method for determining the susceptibility of an at least partially atrophied thymus to reactivation in a patient, comprising:

monitoring the level of TRECs in the patient's blood or serum,

disrupting sex steroid-mediated signaling to the thymus of the patient,

monitoring the level of the TRECs in the patient's blood or serum, and

comparing the level of the TRECs in the patient's T cells before and after disruption of sex steroid-mediated signaling,

wherein an early increase in the level of the TRECs following disruption of sex steroid-mediated signaling indicates susceptibility of the patient's thymus to reactivation.

5 66. The method of claim 65, wherein the TREC levels are monitored by a method comprising:

purifying the patient's T cells,

isolating DNA from the purified T cells, and

10 performing real-time polymerase chain reaction on the isolated DNA with TREC-specific primers and a molecular beacon,

wherein the primers amplify the TREC DNA, and wherein the molecular beacon detects the amplified TREC DNA.

67. The method of claim 66, wherein the TREC-specific primers are selected from the group consisting of SEQ ID NO:1, SEQ ID. NO:2, SEQ ID NO:3, and SEQ ID NO:4.

15 68. The method of claim 65, wherein the patient has a disease that at least in part atrophied the thymus of the patient.

69. The method of claim 65, wherein the patient has had a treatment of a disease, wherein the treatment at least in part atrophied the thymus of the patient.

20 70. The method of claim 69, wherein the treatment is immunosuppression, chemotherapy, or radiation treatment.

71. The method of claim 65, wherein the patient is post-pubertal.

72. The method of claim 65, wherein the sex steroid-mediated signaling to the thymus is disrupted by surgical castration.

73. The method of claim 65, wherein the sex steroid-mediated signaling to the thymus is disrupted by chemical castration.

74. The method of claim 65, wherein the sex steroid-mediated signaling to the thymus is disrupted by administration of one or more pharmaceuticals.

5 75. The method of claim 74, wherein the one or more pharmaceuticals is selected from the group consisting of LHRH agonists, LHRH antagonists, anti-LHRH vaccines, anti-androgens, anti-estrogens, SERMs, SARMs, SPRMs, ERDs, aromatase inhibitors, anti-progestogens, and combinations thereof.

10 76. The method of claim 75, wherein the LHRH agonists are selected from the group selected from the group consisting of Eulexin, Goserelin, Leuprolide, Dioxalan derivatives, Triptorelin, Meterelin, Buserelin, Histrelin, Nafarelin, Lutrelin, Leuprorelin, Deslorelin, Cystorelin, Decapeptyl, Gonadorelin, and combinations thereof.

77. The method of claim 75, wherein the LHRH antagonists are selected from the group consisting of Abarelix, Cetrorelix, and combinations thereof.

15 78. The method of claim 74, wherein the one or more pharmaceuticals are a combination of LHRH agonists and LHRH antagonists.

79. The method of claim 65, wherein the early increase occurs within four weeks following disruption of sex steroid-mediated signaling.

20 80. The method of claim 65, wherein the early increase occurs within two weeks following disruption of sex steroid-mediated signaling.

81. The method of claim 65, wherein the early increase occurs within one week following disruption of sex steroid-mediated signaling.

82. The method of claim 65, wherein the early increase occurs within about 4 to 5 days following disruption of sex steroid-mediated signaling.

83. The method of claim 65, wherein the early increase occurs within about 2 to 3 days following disruption of sex steroid-mediated signaling.

84. The method of claim 65, wherein the early increase occurs within about 24 hours following disruption of sex steroid-mediated signaling.

5 85. A method for delivering a sex steroid analog to a patient, comprising:

laser-irradiating the skin of the patient to create perforations or alterations in the skin,
and

placing the sex steroid analog on the irradiated skin,

wherein the sex steroid analog is delivered through the perforations or alterations in the
10 irradiated skin.

86. A method for delivering a sex steroid analog to a patient, comprising:

delivering the sex steroid analog to the skin of the patient, and

permeabilizing the skin of the patient with high pressure impulse transients,

wherein the impulse transients cause the sex steroid analog to diffuse through the
15 permeabilized skin of the patient.

87. A method for enhancing transplantation of donor hematopoietic stem cells into the thymus of a recipient patient, comprising:

depleting the T cells of the patient,

reactivating the thymus of the patient, and

20 transplanting donor hematopoietic stem cells to the patient,

wherein uptake of the donor hematopoietic stem cells into the patient's thymus is enhanced as compared to the uptake that would have otherwise occurred in a patient prior to thymus reactivation.

88. A method for increasing virus-specific peripheral T cell responsiveness of a patient with an at least partially atrophied thymus, comprising:

reactivating the thymus of the patient,

exposing the patient to a virus,

5 determining the virus-specific peripheral T cell responsiveness in the patient,

wherein the patient has an increased viral-specific peripheral T cell responsiveness as compared to the responsiveness that would have otherwise occurred in a patient prior to thymus reactivation.

89. A method for determining the susceptibility of an at least partially atrophied
10 thymus to reactivation in a patient, comprising:

disrupting sex steroid-mediated signaling to the thymus of the patient, and

monitoring the level in the patient's blood or serum of one or more markers associated with activation of the thymus;

15 wherein an early increase in the level of any one of the markers following disruption of sex steroid-mediated signaling indicates susceptibility of the patient's thymus to reactivation.

90. A method for determining the susceptibility of an at least partially atrophied thymus to reactivation in a patient, comprising:

disrupting sex steroid-mediated signaling to the thymus of the patient, and

20 monitoring the *in vitro* proliferative responsiveness of the T cells in the patient's blood or serum;

wherein an early increase in the *in vitro* proliferative responsiveness of the T cells following disruption of sex steroid-mediated signaling indicates susceptibility of the patient's thymus to reactivation.

91. A method for determining the susceptibility of an at least partially atrophied thymus to reactivation in a patient, comprising:

disrupting sex steroid-mediated signaling to the thymus of the patient, and

5 monitoring the *in vitro* responsiveness of the T cells in the patient's blood or serum to anti-CD3 cross-linking, wherein responsiveness is determined by monitoring proliferation of the T cells;

wherein an early increase in the *in vitro* responsiveness of the T cells to anti-CD3 cross-linking following disruption of sex steroid-mediated signaling indicates susceptibility of the patient's thymus to reactivation.

10 92. A method for determining the susceptibility of an at least partially atrophied thymus to reactivation in a patient, comprising:

disrupting sex steroid-mediated signaling to the thymus of the patient, and

monitoring the level of newly produced T cells in the patient's blood or serum;

15 wherein an early increase in the level of the newly produced T cells following disruption of sex steroid-mediated signaling indicates susceptibility of the patient's thymus to reactivation.

93. A method for determining the susceptibility of an at least partially atrophied thymus to reactivation in a patient, comprising:

disrupting sex steroid-mediated signaling to the thymus of the patient, and

monitoring the level of the TRECs in the patient's blood or serum;

20 wherein an early increase in the level of the TRECs following disruption of sex steroid-mediated signaling indicates susceptibility of the patient's thymus to reactivation.

94. A method for determining the susceptibility of an at least partially atrophied thymus to reactivation in a patient, comprising:

disrupting sex steroid-mediated signaling to the thymus of the patient, and

monitoring the intracellular cytokine levels in the T cells in the patient's blood or serum;

wherein an early increase in the intracellular cytokine levels in the T cells following
disruption of sex steroid-mediated signaling indicates susceptibility of the patient's thymus to
5 reactivation.

95. A method for determining the susceptibility of an at least partially atrophied
thymus to reactivation in a patient, comprising:

monitoring the intracellular cytokine levels in the T cells in the patient's blood or serum,

disrupting sex steroid-mediated signaling to the thymus of the patient,

10 monitoring the intracellular cytokine levels in the T cells in the patient's blood or serum,
and

comparing the intracellular cytokine levels in the T cells in the patient's blood or serum
before and after disruption of sex steroid-mediated signaling;

15 wherein an early increase in the intracellular cytokine levels in the T cells following
disruption of sex steroid-mediated signaling indicates susceptibility of the patient's thymus to
reactivation.

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